Normalization of Fasting Glycaemia by Intravenous GLP-1 ([7-36 Amide] or [7-37]) in Type 2 Diabetic Patients

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Intravenous GLP-1 [7-36 amide] can normalize fasting hyperglycaemia in Type 2 diabetic patients. Whether GLP-1 [7-37] has similar effects and how quickly plasma glucose concentrations revert to hyperglycaemia after stopping GLP-1 is not known. Therefore, 8 patients with Type 2 diabetes (5 female, 3 male; 65 ± 6 years; BMI 34.3 ± 7.9 kg m^{-2}; HbA_{1c} 9.6 ± 1.2 %; treatment with diet alone ($n = 2$), sulphonylurea ($n = 5$), metformin ($n = 1$)) were examined twice in randomized order. GLP-1 [7-36 amide] or [7-37] (1 pmol kg^{-1}min^{-1}) were infused intravenously over 4 h in fasted subjects. Plasma glucose (glucose-oxidase), insulin and C-peptide (ELISA) was measured during infusion and for 4 h thereafter. Indirect calorimetry was performed. Fasting hyperglycaemia was 11.7 ± 0.9 [7-36 amide] and 11.3 ± 0.9 mmol l^{-1} [7-37]. GLP-1 infusions stimulated insulin secretion approximately 3-fold (insulin peak 168 ± 32 and 156 ± 47 pmol l^{-1}, $p < 0.0001$ vs basal; C-peptide peak 2.32 ± 0.28 and 2.34 ± 0.43 nmol l^{-1}, $p < 0.0001$, respectively, with GLP-1 [7-36 amide] and [7-37]). Four hours of GLP-1 infusion reduced plasma glucose (4.8 ± 0.4 and 4.6 ± 0.3 mmol l^{-1}, $p < 0.0001$ vs basal values), and it remained in the non-diabetic fasting range after a further 4 h (5.1 ± 0.4 and 5.3 ± 0.4 mmol l^{-1}, for GLP [7-36 amide] and [7-37], respectively). There were no significant differences between GLP-1 [7-36 amide] and [7-37] (glucose, $p = 0.99$; insulin, $p = 0.99$; C-peptide, $p = 0.99$). Neither glucose oxidation nor lipid oxidation (or any other parameters determined by indirect calorimetry) changed during or after the administration of exogenous GLP-1. In conclusion, GLP-1 [7-36 amide] and [7-37] normalize fasting hyperglycaemia in Type 2 diabetic patients. Diabetes therapy (diet, sulphonyl ureas or metformin) does not appear to influence this effect. In fasting and resting patients, the effect persists during administration of GLP-1 and for at least 4 h thereafter, without rebound. Significant changes in circulating substrate concentrations (e.g. glucose) are not accompanied by changes in intracellular substrate metabolism.

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Introduction

Glucagon-like peptide 1 [7-36 amide] (GLP-1) is an insulinotropic ('incretin') and glucagonostatic hormone produced in and secreted from enteroglucagon-producing L cells mainly located in the ileum, colon, and rectum.[1–4] After meals, together with Gastric Inhibitory Polypeptide (GIP), it contributes to the postprandial insulin response.[5] In pharmacological concentrations, approximately 3–4 fold higher than typical postprandial values, exogenous GLP-1 [7-36 amide] stimulates insulin, lowered glucagon, and normalized glucose concentrations in hyperglycaemic Type 2 diabetic patients.[6] Therefore, GLP-1 has therapeutic potential.[3,4,7]

A second molecular form of GLP-1, sequence [7-37] ('glycine extended GLP-1'),[2,8] is cosecreted with GLP-1 [7-36 amide].[9] For reasons that are incompletely understood, 'glycine-extended GLP-1' escapes the carboxy-terminal amidation. In the basal state, low concentrations of both molecular forms of GLP-1 circulate, whereas after meal stimulation, 'amidated' GLP-1 rises more prominently.[9] GLP-1 [7-37] has similar effects in normal subjects in the basal state,[10] when—due to their glucose dependence[11,12]—only small effects are expected and is reported to enhance insulin secretion in hyperglycaemic Type 2 diabetic patients.[13] A direct comparison of effects of GLP-1 [7-36 amide] and [7-37] in Type 2 diabetic patients has not been reported. Furthermore, the potential for rebound hyperglycaemia after stopping intravenous administration of GLP-1, or whether the

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profound changes in plasma substrate concentrations induced by GLP-1 are accompanied by changes in intracellular substrate metabolism, are not known.

It was the aim of the present study to compare effects of exogenous GLP-1 [7-36 amide] versus [7-37] on insulin and glucagon secretion in fasting Type 2 diabetic patients. The observation period was chosen to detect changes in glycaemia, insulin and glucagon secretion after stopping the administration of GLP-1, especially since in fed patients the discontinuation of GLP-1 administration leads to some 'rebound' increment.[13] Last, in the basal state, during the administration of GLP-1 and at the end of the study period indirect calorimetry[14] was performed to detect changes in intracellular substrate metabolism induced by GLP-1 ([7-36 amide] or [7-37]). Preliminary results have been reported in abstract form.[15]

Patients and Methods

The study protocol was approved by the ethics committee of the medical faculty of the Ruhr-University, Bochum, on 21 October 1993. Written informed consent was obtained from all participants.

Subjects

Eight Type 2 diabetic patients were studied (Table 1). A wide range of diabetes duration (from newly diagnosed to 22 years), adiposity (body mass index from 22.2 to 46.1 kg m^{-2}), and treatment modality (diet alone, diet plus sulphonylurea or diet plus metformin) was included. Metabolic control also varied (from HbA_{1c} 6.8 to 11.4 %). Plasma cholesterol was >240 mg dl^{-1} in 5 patients, triglycerides were >200 mg dl^{-1} in 4 patients. Macrovascular disease was documented in 4 patients.

Study Design

All participants were studied in the morning (after an overnight fast), in random order, on two occasions: either GLP-1 [7-36 amide] or GLP-1 [7-37] was administered intravenously at an infusion rate of 1 pmol kg^{-1} min^{-1} for 240 min. The dosage was slightly lower than in a previous study with GLP-1 [7-36 amide] (1.2 pmol kg^{-1} min^{-1}) that had shown a complete normalization of fasting plasma glucose in Type 2 diabetic patients with secondary failure to sulphonylurea treatment.[6] Since this previous study had demonstrated a clear difference to the administration of placebo (0.9 % NaCl with 1 % human serum albumin, Human-Albumin 20 % Behring, salzarm, Behringwerke AG, Marburg, FRG), no comparison with placebo was made in the present study.

Diabetes medication was administered until the evening before the study days and discontinued during the experiments, but antihypertensive medication (enalapril and nifedipine in one patient each) and isosorbitol mononitrate (in one patient) was given at the time of the experiments. One day was allowed between the two experiments, with usual eating pattern and medication (including sulphonylurea and metformin). Two forearm veins were cannulated (Moskito 123, 18 gauge, Vygon, Aachen, FRG) for blood sampling and for GLP-1 administration. Patency was ensured using 0.9% NaCl.

After drawing basal blood specimens, at 0 min, the infusion of GLP-1 ([7-36 amide] or [7-37]) was started. Blood was drawn at the time points indicated in Figures 1, 2, 3, and 4 for 480 min (i.e. for an additional 240 min after discontinuing the intravenous administration of GLP-1) and plasma glucose was determined immediately.

Blood was drawn into tubes containing EDTA and aprotinin (Trasylol; 20 000 KIU ml^{-1}, 200 μl per 10 ml blood; Bayer AG, Leverkusen). A sample (approximately 100 μl) was stored in NaF (Microvette CB 300; Sarstedt, Nümbrecht, FRG) for the measurement of glucose. After centrifugation, plasma for hormone analyses was kept frozen at −30 °C.

In the basal state (−25 to 0 min), at the end of the GLP-1 infusion period (215 to 240 min) and at the end of the observation period (455 to 480 min), a translucent gas-tight hood was placed over the patients' heads. Oxygen consumption and carbon dioxide production was measured using a Deltatrak MBM 100 metabolic monitor (Datex, obtained from Hoyer-Engström, Bremen, FRG). Respiratory quotients, glucose and lipid oxidation rates, and energy expenditure were calculated according to standard equations.[14] Protein oxidation was calculated for all patients from nitrogen (urea) excretion in urine specimens collected over 24 h on an occasion different from the experimental situation, but was assumed to remain constant over the study period.

Peptides

Synthetic GLP-1 [7-36 amide] and GLP-1 [7-37] were purchased from Saxon Biochemicals GmbH, Hannover, FRG. The lot number of GLP-1 [7-36 amide] (pharmaceutical grade) was PGAS 242, FGLP7369301 A, net peptide content 88 %. The lot number for GLP-1 [7-37] was PGAS 243, Lot ZJ 222, net peptide content 91 %. The peptides were dissolved in 0.9 % NaCl/1 % human serum albumin, filtered through 0.2 μm nitrocellulose filters (Millipore, Bedford, Mass., USA) and stored frozen at −30 °C as previously described. HPLC profiles (provided by the manufacturer) showed that the preparation was >99 % pure (single peak co-eluting with appropriate standards). Samples were analysed for bacterial growth (standard culture techniques) and for pyrogens (*Limulus* amebocyte lysate endo-LAL, Chromogenix AB, Mölndal, Sweden). No bacterial contamination was detected. Endotoxin concentrations in the GLP-1 stem solutions always were <0.03 EU ml^{-1}.

Laboratory Determinations

Glucose was measured using a glucose oxidase method with a Glucose Analyser 2 (Beckman Instruments, Mun-

Table 1. Patient characteristics of non-insulin-dependent (Type 2) diabetic patients participating in the study

Patient number	Sex (M/F)	Age (yr)	Body mass index (kg m^{-2})	Waist–hip ratio	Duration of diabetes (yr)	Glycated haemoglobin (%)[a]	Diabetes therapy[b]	Creatinine clearance (ml min^{-1})	RR (mmHg)	Retinopathy	Nephropathy	Neuropathy	Macroangiopathy[c]
1	F	74	37.5	1.06	0	9.9	D	64	140/80	no	no	no	CVI
2	F	69	40.0	1.07	12	10.2	D,S	67	130/75	no	no	no	no
3	M	67	22.2	0.99	3	8.9	D,S	69	140/80	no	no	yes	CHD, CVI
4	M	64	41.0	0.86	2	6.8	D	103	180/110	no	no	no	no
5	F	64	30.1	1.00	16	10.0	D,S	79	130/70	no	no	no	no
6	F	53	46.1	1.02	13	10.0	D,M	52	130/75	no	yes	yes	PVI, CVI
7	F	63	33.2	1.04	14	11.4	D,S	60	160/80	no	no	no	no
8	M	67	24.0	0.93	22	9.5	D,S	88	130/70	no	no	no	PVI
Mean	5 F	65	34.3	1.00	10	9.6	5 S	73	143/80	8 no	7 no	6 no	4 no
SD	3 F	6	7.9	0.07	7	1.3	1 M	16	17/12	–	1 yes	2 yes	4 yes

[a]Normal values: 4.2–6.3 %.
[b]D, diet; M, metformin; S, sulfonylurea.
[c]CHD, coronary heart disease; CVI, cerebrovascular insufficiency; PVI, peripheral vascular insufficiency.

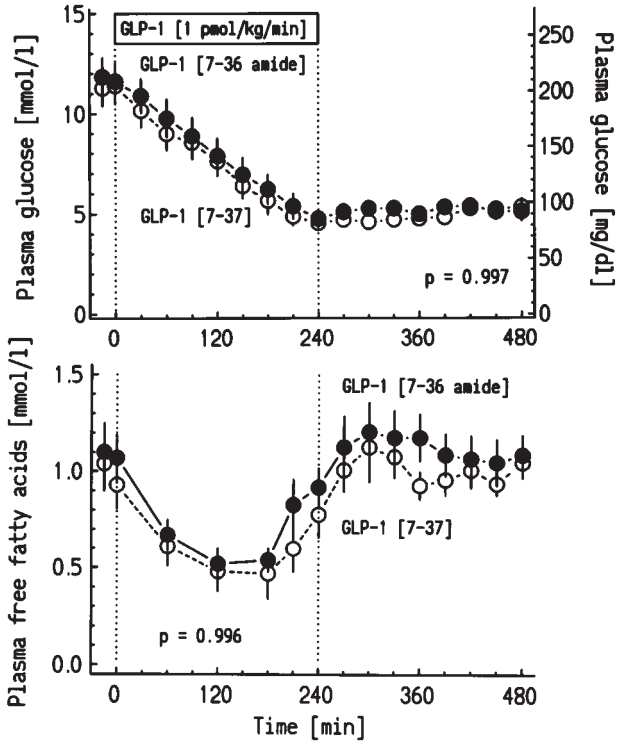

Figure 1. Plasma concentrations of (a) glucose and (b) free fatty acids during the intravenous infusion of GLP-1 ([7-36 amide] or [7-37]), 1.0 pmol kg^{-1} min^{-1}, in 8 Type 2 diabetic patients. Closed symbols: GLP-1 [7-36 amide]; open symbols: GLP-1 [7-37]. Mean ± SEM; *p*-values represent the interaction of experiment (GLP-1 [7-36 amide] versus [7-37]) and time as calculated by repeated-measures analysis of variance (RM-ANOVA). Asterisks indicate significant differences ($p < 0.05$ by Student's *t*-test) at individual time points

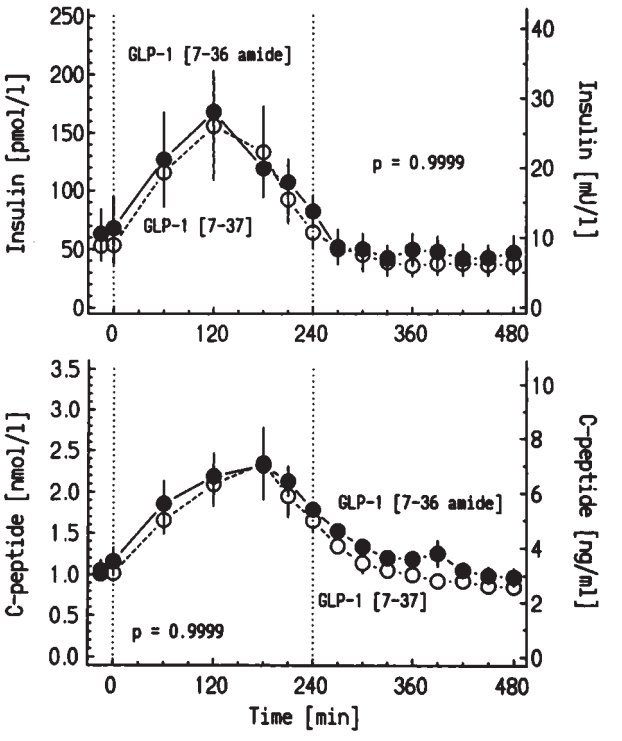

Figure 2. Plasma concentrations of (a) insulin and (b) C-peptide during the intravenous infusion of GLP-1 ([7-36 amide] or [7-37]), 1.0 pmol kg^{-1} min^{-1}, in 8 Type 2-diabetic patients. Closed symbols: GLP-1 [7-36 amide]; open symbols: GLP-1 [7-37]. Mean ± SEM; *p*-values represent the interaction of experiment (GLP-1 [7-36 amide] versus [7-37]) and time as calculated by repeated-measures analysis of variance (RM-ANOVA). Asterisks indicate significant differences ($p < 0.05$ by Student's *t*-test) at individual time points

ich, FRG). Plasma IR-insulin and C-peptide were determined using commercial immunoassay kits. Insulin was measured using an insulin microparticle enzyme immunoassay (MEIA; IMx Insulin, Abbott Laboratories, Wiesbaden, FRG). Intra-assay coefficients of variation were <4 %. C-peptide was measured using C-peptide-antibody-coated microtitre wells (C-peptide MTPL EIA; DRG Instruments GmbH, Marburg, FRG). Intra-assay coefficients of variation were <6 %. Human insulin and C-peptide were used as standards.

IR-GLP-1 was determined in ethanol-extracted plasma as previously described,[10,16] using antiserum 89 390 (final dilution 1:150 000) for experiments with exogenous GLP-1 [7-36 amide], and synthetic GLP-1 [7-36 amide] for tracer preparation and as standard. Recovery of GLP-1 [7-36 amide] standards after alcohol extraction was 75 ± 8 %. The experimental detection limit (2 standard deviations over samples not containing GLP-1 [7-36 amide]) was <5 pmol l^{-1}. Antiserum 89 390 binds to the amidated carboxy-terminus of GLP-1 [7-36 amide]. Both when GLP-1 [7-36 amide] or GLP-1 [7-37] were infused, measurements were performed with a non-discriminating, side-viewing antibody (antiserum 2135, final dilution 1:150 000), using GLP-1 [7-36 amide] or [7-37] as standard, as appropriate. This antibody reacts with equal affinity with both GLP-1 [7-36 amide] and [7-37][17] and was used to normalize the steady state concentrations of the two molecular forms of GLP-1. Recovery after alcohol extraction for GLP-1 [7-37] similarly was ≅75 %. Intra-assay coefficients of variation for the antiserum 2135 assay were <8 %.

Pancreatic glucagon was assayed in ethanol-extracted plasma using antibody 4305.[18] Plasma free fatty acids (NEFA) were assayed using reagents from Wako chemicals (Neuss, FRG) on a Hitachi 709 autoanalyser. Triglycerides and total cholesterol was measured using standard clinical chemistry. Each patient's set of plasma samples was assayed at the same time to avoid errors due to inter-assay variation.

Statistical Analysis

Results are reported as mean ± SEM. All statistical calculations were carried out using repeated-measures analysis of variance (ANOVA) using NCSS Version 5.01 (Jerry Hintze, Kaysville, Utah, USA). If a significant interaction of treatment and time was documented ($p < 0.05$), values at single time points were compared by Student's *t*-test (paired analyses). The p-values were corrected for the number of comparisons made according

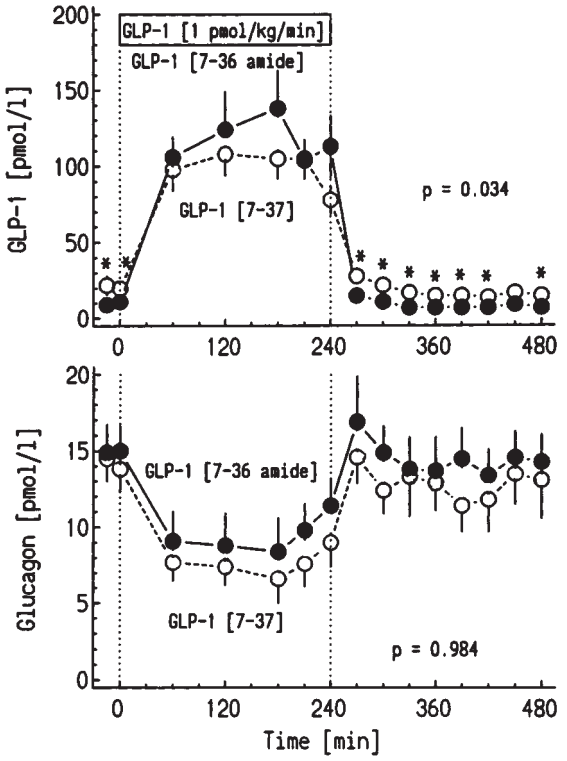

Figure 3. Plasma concentrations of (a) GLP-1 and (b) glucagon during the intravenous infusion of GLP-1 ([7-36 amide] or [7-37]), 1.0 pmol kg^{-1} min^{-1}, in 8 Type 2-diabetic patients. Closed symbols: GLP-1 [7-36 amide]; open symbols: GLP-1 [7-37]. Mean ± SEM; *p*-values represent the interaction of experiment (GLP-1 [7-36 amide] versus [7-37]) and time as calculated by repeated-measures analysis of variance (RM-ANOVA). Asterisks indicate significant differences ($p < 0.05$ by Student's *t*-test) at individual time points

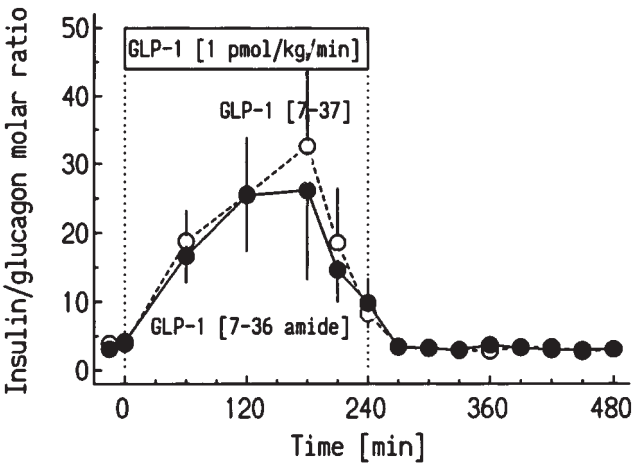

Figure 4. Molar ratio of insulin/glucagon in peripheral venous plasma during the intravenous infusion of GLP-1 ([7-36 amide] or [7-37]), 1.0 pmol kg^{-1} min^{-1}, in 8 Type 2-diabetic patients. Closed symbols: GLP-1 [7-36 amide]; open symbols: GLP-1 [7-37]. Mean ± SEM; *p*-values represent the interaction of experiment (GLP-1 [7-36 amide] versus [7-37]) and time as calculated by repeated-measures analysis of variance (RM-ANOVA)

to Bonferroni-Holm. A corrected two-sided *p*-value <0.05 was taken to indicate significant differences.

Results

Plasma Glucose

Basal plasma glucose concentrations were 11.7 ± 0.9 mmol l^{-1} (before GLP-1 [7-36 amide]) and 11.3 ± 0.9 mmol l^{-1} (before GLP-1 [7-37]); 1 pmol kg^{-1} min^{-1} GLP-1 of either molecular form reduced plasma glucose concentrations to 4.8 ± 0.4 mmol l^{-1} (GLP-1 [7-36 amide]) and 4.6 ± 0.3 mmol l^{-1} (GLP-1 [7-37]). Changes with time were significant (RM-ANOVA, $p < 0.0001$). By paired *t*-tests, in both experiments, plasma glucose concentrations at the end of the infusions were significantly lower ($p < 0.0001$) than in the basal state. Even 4 h after the termination of the GLP-1 infusion, glucose remained low ($p < 0.0001$ vs basal) and in the normal fasting range (5.1 ± 0.4 mmol l^{-1} [7-36 amide]; 5.3 ± 0.4 mmol l^{-1} [7-37]; $p = 0.69$ for the comparison by paired *t*-test). By RM-ANOVA, there was no difference between experiments (GLP-1 [7-36 amide] vs [7-37], $p = 0.52$), nor was there a significant interaction between time and experiment ($p = 0.99$; Figure 1 (a)).

Plasma Insulin and C-peptide

Basal plasma insulin concentrations (Figure 2 (a)) were 66 ± 24 pmol l^{-1} (before GLP-1 [7-36 amide]) and 53 ± 15 pmol l^{-1} (before GLP-1 [7-37]); 1 pmol kg^{-1} min^{-1} GLP-1 of either molecular form elevated insulin concentrations to 168 ± 32 pmol l^{-1} and 156 ± 47 pmol l^{-1}, respectively, at 120 min. However, during the ongoing administration of GLP-1, insulin concentrations returned to basal values when plasma glucose concentrations (Figure 1 (a)) approached the normal fasting range. Changes with time were significant (RM-ANOVA, $p < 0.0001$). Four hours following the infusions, plasma insulin concentrations remained in the normal fasting range (46 ± 14 mmol l^{-1} [7–36 amide]; 37 ± 9 mmol l^{-1} [7–37]; $p = 0.10$, paired *t*-test). There was a trend towards lower insulin ($p = 0.12$ and 0.04 for GLP-1 [7-36 amide] and [7-37], respectively) and C-peptide ($p = 0.08$ and 0.02 for GLP-1 [7-36 amide] and [7-37], respectively) concentrations at the end of the experiments in comparison to the basal state. By RM-ANOVA, there was no difference between experiments ($p = 0.71$), nor a significant interaction between time and experiment ($p = 0.99$).

Plasma C-peptide concentrations paralleled those of insulin (Figure 2 (b)). Changes with time were significant (RM-ANOVA, $p < 0.0001$). There was no difference between experiments ($p = 0.39$), nor a significant interaction between time and experiment ($p = 0.99$).

GLP-1 Concentrations

GLP-1 infusion raised plasma levels by approximately 100 pmol l^{-1} (Figure 3 (a)), with no significant differences between the two forms ($p = 0.10$ to 0.86). There were apparent differences in the basal state (Figure 3 (a)), asterisks), probably due to the correction of GLP-1 concentrations for the assay characteristics the GLP-1 [7-37] determination using a 'side-viewing' antiserum (2135).[10,17] After the discontinuation of GLP-1 infusions, basal concentrations were reached again within 30 min.

Changes with time were significant (RM-ANOVA, $p < 0.0001$). There was no difference between experiments ($p = 0.90$), but a significant interaction between time and experiment ($p = 0.03$).

Glucagon Concentrations

Basal plasma glucagon concentrations (Figure 3 (b)) were 15 ± 2 pmol l^{-1} (before GLP-1 [7-36 amide]) and 14 ± 2 pmol l^{-1} (before GLP-1 [7-37]); 1 pmol kg^{-1} min^{-1} GLP-1 of either molecular form lowered glucagon concentrations to 8 ± 2 pmol l^{-1} and 7 ± 2 pmol l^{-1}, respectively, at 180 min. During continued administration of GLP-1, glucagon concentrations rose towards basal values again as plasma glucose (Figure 1 (a)) approached the normal fasting range. Changes with time were significant (RM-ANOVA, $p < 0.0001$). There was no difference between experiments ($p = 0.38$), nor a significant interaction between time and experiment ($p = 0.98$). The molar ratio of insulin and glucagon concentrations was thus transiently raised by the infusion of GLP-1 from basal values ≈4 to ≈30 (i.e. approximately seven-fold; Figure 3). Changes with time were significant (RM-ANOVA, $p < 0.0001$). There was no difference between experiments ($p = 0.94$), nor a significant interaction between time and experiment ($p = 1.00$).

Free Fatty Acids, Triglycerides, Cholesterol

The concentration of non-esterified fatty acids was significantly reduced, both with GLP-1 [7-36 amide] or [7-37] (Figure 1 (b)), returning to basal values when plasma glucose concentrations approached the normal fasting concentration range. Changes with time were significant ($p < 0.0001$), whereas there were no significant differences between the two forms of GLP-1 ($p = 0.35$) nor in the interaction of experiment and time ($p = 1.00$). Plasma triglycerides fell over time ($p = 0.018$ by RM-ANOVA), with no significant difference between experiments ($p = 0.24$) or in the interaction between time and experiment ($p = 0.99$). Cholesterol concentrations did not change (experiment: $p = 0.35$; time: $p = 0.12$; interaction: $p = 0.47$).

Indirect Calorimetry

As shown in Figure 5, none of the parameters measured by indirect calorimetry changed significantly, either by the end of the GLP-1 infusion period or during the subsequent 4 h.

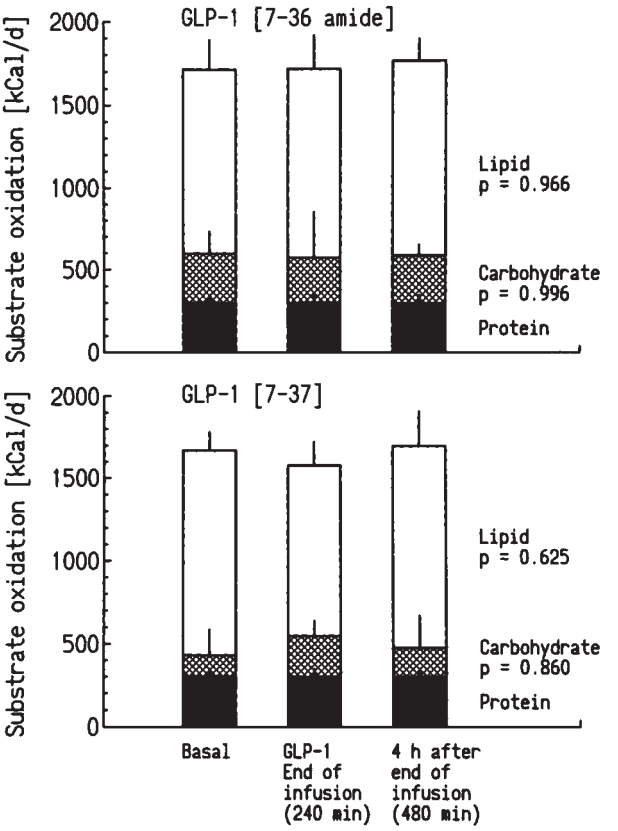

Figure 5. Caloric equivalents of protein oxidation (calculated from urinary nitrogen excretion, black columns), glucose oxidation (hatched columns), and lipid oxidation (open columns) in the basal state, at the end of the 240 min infusion period for GLP-1 ((a): [7-36 amide] (b): [7-37]), and 240 min after stopping the infusion of GLP-1. Mean ± SEM; *p*-values represent one-way ANOVA (analysing changes in the time course of experiments)

Discussion

The present study extends our previous finding that GLP-1 [7-36 amide] can normalize fasting plasma glucose in people with diabetes mellitus.[6,19] GLP-1 [7-37] ('glycine-extended GLP-1'), a less abundant molecular variant of GLP-1,[9] proved equally active in raising insulin, lowering glucagon and normalizing glucose concentrations in Type 2 diabetic patients as GLP-1 [7-36 amide]. Similar findings have been reported in animal experiments[20] and in normal subjects,[10] although in the latter, only a minor stimulation of insulin secretion is expected due to the glucose dependence of GLP-1 effects on β cells.[11,12] The 'antidiabetogenic' action[6,7,13,21] of GLP-1 [7-36 amide] and [7-37] in hyperglycaemic Type 2 diabetic patients is of interest as GLP-1 or its analogues might in future broaden the therapeutic armamentarium.[7] GLP-1 [7-36 amide] and [7-37] both appear to be suitable mother compounds for this purpose. This is in line with structure–activity analyses indicating that modifications of the

N-terminus are associated with greater variations in biological potency of GLP-1-like molecules than are introduced by changes in the C-terminus.[22–24]

We have not compared GLP-1 to placebo in this study as previously, the administration of vehicle led only to a slight reduction in fasting glycaemia,[6] and fasting plasma glucose concentrations, while the effects of GLP-1 [7-36 amide] were similar to the present results. A *post hoc* statistical analysis comparing the present responses of glucose concentrations with the previously published control group[6] shows that both molecular variants of GLP-1 were significantly more potent than placebo (experiment: $p = 0.02$; interaction of experiment and time: $p < 0.0001$) from 120 to 240 min after starting the infusion ($p < 0.05$ by *t*-test).

The glucose dependency of the insulinotropic effects of GLP-1 is evident from insulin and C-peptide responses to the administration of GLP-1: insulin (and C-peptide) were stimulated only during hyperglycaemia and returned to normal as normal fasting glucose concentration range was approached, despite ongoing infusion of GLP-1. None of the patients therefore became hypoglycaemic during GLP-1 administration. The glycaemic threshold for the insulinotropic action of GLP-1 is similar in patients with Type 2 diabetes (this study and the studies of Nauck *et al.*[6,19]) and normal subjects,[5,12,25] and differences in glycaemic control, degree of overweight and pretreatment of Type 2 diabetic patients are not associated with differences in the response towards GLP-1, especially not with differences in glucose thresholds for its effect. The glucagon response also appears to be glucose-dependent, as was seen in our previous study.[6]

The effects of GLP-1 infusion on glucose, insulin, C-peptide and glucagon persisted for 4 h at least, although GLP-1 plasma concentrations fell immediately after stopping the infusion. At this time, normal fasting plasma glucose concentrations were maintained at approximately the same insulin and glucagon concentrations that were measured during basal hyperglycaemia. The most likely explanation for this discrepancy is that normoglycaemia following GLP-1 infusion is not the immediate consequence of insulin and glucagon concentrations but of the changes that occurred during the infusion, including stimulated insulin and lowered glucagon concentrations, and the elevation of the insulin/glucagon molar ratio. Fasting glycaemia in Type 2 diabetes is largely determined by hepatic glucose output,[26] including both glycogenolysis and gluconeogenesis.[27,28] The activity of these metabolic pathways, especially gluconeogenesis, is influenced by the changes in circulating insulin, glucagon and non-esterified fatty acid concentrations,[29] secondary to the GLP-1. Immediate regulation of enzymes such as glycogen synthase and glycogen phosphorylase by insulin and glucagon,[30] may be followed by a lasting effect due to enzyme induction or enzyme repression. Since the period of GLP-1 administration was 4 h, only enzymes with a short half-life are candidates for the prolonged effect, e.g. phosphoenolpyruvate carboxykinase (PEPCK, as the rate limiting, 'key' enzyme of gluconeogenesis[31]), which shows a marked change in enzyme activity within 4 h of exposure to altered concentrations of insulin or glucagon, or glucokinase, the rate-limiting enzyme of glycolysis, the synthesis of which is induced by insulin and repressed by glucagon.[32] This hypothesis might explain why, after the exposure to GLP-1, lower glucose concentrations were maintained by insulin levels which tended to be lower than in the basal state (Figure 2). An alternative view is that lower glucose concentrations were able to maintain similar insulin and C-peptide concentrations after the exposure to GLP-1 because of a 'priming effect' for the exposure of pancreatic β cells to GLP-1.[33] If so, the duration of the B cell 'memory' is 4 h or longer.

Insulin administration is unlikely to have so prolonged a hypoglycaemic effect as well. The 'off response' of insulin action has been studied in detail. After stopping insulin administrations leading to plasma concentrations $\approx$120 or 350 mU l^{-1}, the stimulation of glucose disposal and the suppression of hepatic glucose output were no longer observed after 75 and 150 min, respectively.[34,35] These insulin concentrations were higher than those achieved in our study, and a higher insulin dose has a more prolonged post-infusion action. It therefore appears unlikely that the dissipation of insulin action alone seen in our study would not lead to a rise in glycaemia. We conclude that the potential of GLP-1 to maintain normal fasting plasma glucose concentrations after the duration of its administration is relatively specific for the action of GLP-1.

The duration of normalization of glycaemia after GLP-1 infusion needs to be investigated. One interesting concept from the present study is the potential to normalize glycaemia overnight by subcutaneous injections of GLP-1 in the evening.[19] Normalization of glycaemia overnight with intravenous GLP-1,[36,37] reduced mean 24 h glycaemia by approximately 18 %,[36] although it had no persistent effect on day-long glycaemia once its administration was stopped.[36,37] The difference in these studies to our present one is that our subjects remained fasting. The persistent normalization of glycaemia through transient (e.g. 4 h) administrations of GLP-1 may be restricted to resting, fasting patients.

Indirect calorimetry showed no changes in intracellular substrate oxidation rates with GLP-1. This is in contrast to the findings of Shalev *et al.*,[38] who infused GLP-1 [7-37 amide] into healthy volunteers while clamping glucose concentrations at 8 mmol l^{-1}. Under these conditions, insulin was stimulated greatly, to much higher levels than in the present study. This was accompanied by a rise in energy expenditure, in CO_2 production, in oxygen consumption, and in the respiratory quotient.[38] Our failure to find such effects may be that our measurements were not performed at the time of the greatest deviation from the basal glucose/fatty acid ratio or of elevated insulin concentrations but are more likely to be due to the smaller rise in insulin concentrations and the insulin

resistance of our Type 2 diabetic patients. On the other hand, intracellular substrate availability and oxidation may be governed by factors different from those that determine circulating concentrations. The lack of effect of GLP-1 on net hepatic, muscular and adipose tissue handling of intracellular substrates may be taken as additional evidence against a direct effect of GLP-1 on these tissues[39] and by the lack of expression of β cell-like GLP-1 receptors in these tissues.[40,41] Certainly, changes in intracellular substrate oxidation rates do not appear to play a major role in mediating the normalization of plasma glucose concentrations in Type 2 diabetic patients.

In conclusion, both GLP-1 [7-36 amide] and [7-37] normalize fasting plasma glucose concentrations in Type 2 diabetic patients with fasting hyperglycaemia, who had been treated by diet alone or in combination with sulphonylureas or metformin. The normalization of glycaemia outlasts the period of administration of GLP-1 (of either molecular form) by at least 4 h during continued fasting. These profound changes in substrate concentrations (glucose, free fatty acids) are not accompanied by parallel changes in glucose versus lipid oxidation.

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